# Gold-based complexes

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# CHAPTER 7

## SUMMARY, DISCUSSION AND PERSPECTIVES

## 1/ Summary and discussion

After a bibliographic introduction on metal-based anticancer agents, which gives an overview of the recent progress in the field, I have described in this thesis the synthesis and the biological evaluation of several gold-based derivatives as possible anticancer agents.
Specifically, I focused on organometallic complexes. Such class of metal-based complexes is characterized by a metal-carbon bond, that induces peculiar properties compared to classical coordination complexes. Although metal-carbon bonds are usually recognized to be reactive, which is reflected by the use of organometallics for organic synthesis,[1] some particular organometallic complexes including *N*-heterocyclic carbenes (NHC), cyclometalated and alkynyl complexes appear highly stable even in physiological environment.[2] Moreover, the metal-carbon bond is characterized by both a high *trans* effect and *trans* influence that greatly modify the lability of other metal-ligand bonds and so the reactivity of the complexes toward substitution reactions.[3] Such properties can strongly influence the behavior of the compounds in the presence of biological targets (*i.e.* DNA and/or proteins), thus modifying their cytotoxicity and selectivity toward cancer cells. As I reported in **chapter 1**, one of the most interesting properties of NHCs (beyond the ones we discussed previously) is their relative ease of derivatization. This enables chemists to design a very broad range of structures by introducing variations in the NHC backbone, functional groups on nitrogens as wells as ancillary ligand as depicted in Figure 1.

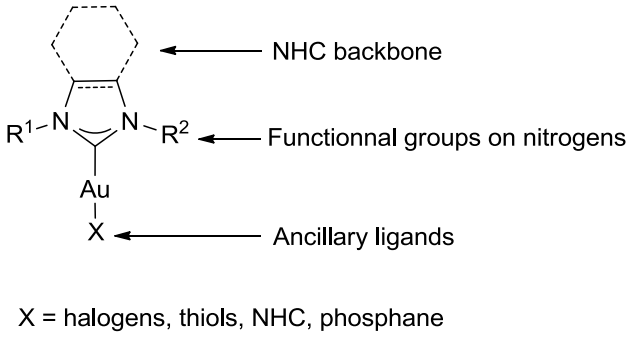



**Figure 1**: General scheme of derivatization possibilities of Au(I)-NHC compounds.

Within this frame, I adopted different synthetic strategies for the synthesis of our gold-based NHC compounds. In **chapter 2** I explored the synthesis of NHC complexes using the natural product caffeine while in **chapter 4** I mainly worked on the ancillary ligand by incorporating chloride- and different sugar moieties to target the compound to cancer cells that overexpress glucose transporters. The most relevant complexes of each series are depicted in Figure 2.

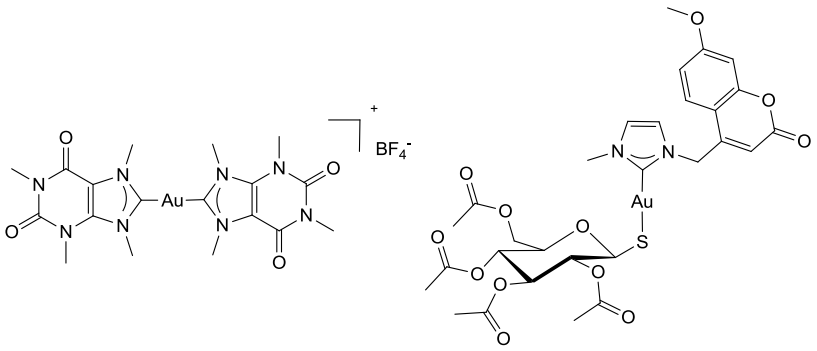



**Figure 2:** Chemical structures of the most representative Au(I)-NHC complexes from chapters 2 and 4, respectively.

In **chapter 2**, I showed that the use of caffeine as a scaffold in gold(I)-NHC complexes, although leading to a lower toxicity compared to the benzimidazole scaffold, could drastically increase the selective toxicity for ovarian cancer cells associated with a lower toxicity for healthy cells and tissues. Additionally, I identified a new potential target of gold-NHC complexes: the G-quadruplex structure of DNA. The stabilization of G-quadruplex of DNA by small molecules is investigated to regulate key cellular events.[4] In particular [TMX-Au-TMX].$BF_4$ (TMX = 1,3,7,9-tetramethylxanthin-8-ylidene) appeared to be highly selective of the G-quadruplex structure compared to any other DNA structures. Although up to now no direct evidence for the involvement of the stabilization of G-quadruplex in the selectivity of [TMX-Au-TMX].$BF_4$ for ovarian cancer cells has been found, these results show the possible target of gold-compounds to DNA.

I also investigated the incorporation of two glucose derivatives already reported to enhance the uptake of gold compounds into cancer cells. Indeed, in **chapter 4**, the grafting of a thio-*β*-D-glucose tetra-acetate moiety was shown to increase the cytotoxicity of the NHC compound compared to its chlorido analog. The equivalent with the unprotected glucose moiety appeared not cytotoxic on the panel of studied cell lines. This last finding ruled out our starting hypothesis concerning a possible targeting of the cancer cells using glucose derivatives via the glucose uptake transporter. Finally, the possible mechanism of action of our Au(I)-NHC compounds was investigated. TrxR, an enzyme involved in the cellular redox balance and overexpressed in cancer cells, is known to be the main target of many gold-based compounds.[5] Biochemical assays suggested that our compounds can inhibit TrxR *via* binding of gold to the selenocysteine residue. The lead compound of that series (fig. 2) could inhibit TrxR in cell lysate suggesting the involvement of the inhibition of TrxR in its cytotoxic activity. However, the best inhibitor of TrxR appeared to the least cytotoxic in the

cancer cells, indicating that inhibition of the TrxR is not the only relevant factor for the cytotoxic activity.

Depending on the type of Au(I)-NHC complexes I could identify two different possible targets: the G-quadruplex structure of DNA for the caffeine-based compounds (chapter 2) and the seleno-enzyme TrxR for the coumarin-based compounds (chapter 4). A very recent study from Ott *et al.* describes the pro-apoptotic pathway of a Au(I)-NHC complex (Figure 3). The authors observed beyond inhibition of the TrxR/Trx system and the increase of oxidative stress, markers for DNA damages as well as inhibition of the p53 transcription factor.[6] This confirms my findings about a broader scope of potential intracellular targets of Au-based compounds.

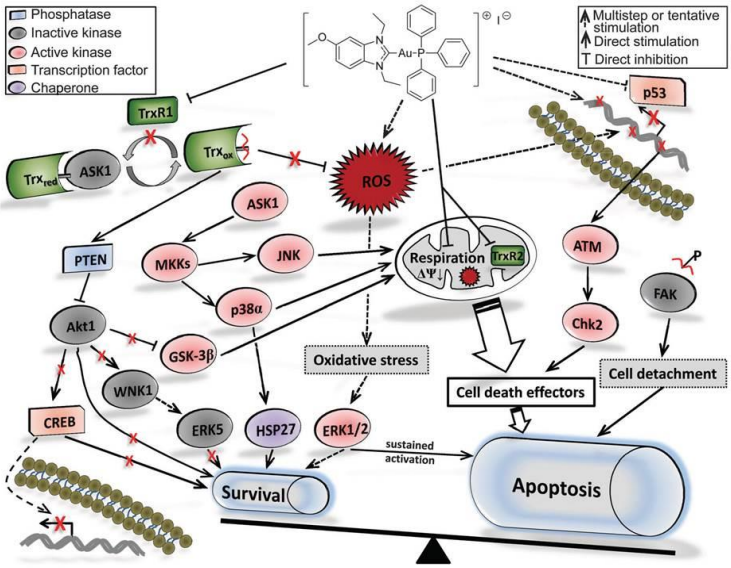
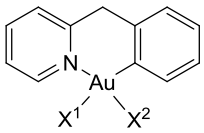



**Figure 3:** Au(I)-NHC-induced cell death pathway. Reproduced from ref. 6.

Overall, more precise determination of the actual mechanism of action of metal-based drugs is still necessary to pursue. In addition to application of these compounds as possible tools to gain insight into the mechanism of action of metal based drugs, their use as *theranostics* (*i.e.* compounds bearing fragment for both therapy and imaging) also appears particularly promising.[7] Indeed, the imaging of metal-based compounds in biological systems could provide very interesting information on the mode of uptake of the compound (by active transport or passive diffusion) as well as on their intracellular distribution.

In **chapter 5**, I explored another class of organometallic complexes (the cyclometalated complexes) and another oxidation state of gold (+III oxidation state). Starting

from the dichlorido (C^N) cyclometalated Au(III) precursor, I synthesized a series of cyclometalated Au(III) with different ancillary ligands including glucose derivatives and phosphane ligands as depicted in Figure 4.




$X^1 = X^2 = Cl$
$X^1 = Cl\ X^2 = PTA$
$X^1 = Cl\ X^2 = PPh_3\text{-Coum}$
$X^1 = Cl\ X^2 = GlucS$
$X^1 = X^2 = GlucS$

**Figure 4:** Structure of the cyclometalated Au(III) complexes studied in chapter 5.

By using NMR spectroscopy ($^1H$, $^{13}C\{^1H\}$ and $^{31}P\{^1H\}$), X-Ray diffraction and Far-IR spectrophotometry techniques in combination, I could assess the structures of the different compounds including the location of the ancillary ligand in *trans* position to the nitrogen. The compounds were screened *in vitro* on a panel of human cancer cells and healthy kidney cells. I noticed an increased cytotoxicity of all new compounds compared to the dichlorido precursor. These results proved the potential of optimization of the scaffold by tuning the ancillary ligands. I could even notice an higher cytotoxicity compared to cisplatin for one phosphane-based compound on the breast cancer cell line. Moreover, as noticed in **chapter 4**, the presence of the thio-glucose moiety did not improve the cytotoxicity on lung cancer cells overexpressing glucose transporters (GLUT-1).

Following the idea of coupling the Au(I)-NHC moiety to other entities for the enhancement of the anticancer properties, I applied in **chapter 3** the concept of multinuclearity to the chemistry of Au(I)-NHC complexes. Indeed a few years ago the increased anticancer properties of polynuclear Ti/Ru and Ti/Au complexes compared to their mononuclear fragments and a mixture of these fragments was demonstrated.[8] I thus decided to couple the Au(I)-NHC moiety to different late metals including gold, ruthenium and copper giving rise to late/late type bimetallic complexes. Due to the close reactivity of the two envisaged metals, the design and the selective coordination of the different metal centers appeared relatively delicate[9] and this justified the development of my post-metalation coupling method. I synthetized two different families of bimetallic complexes by direct coupling of two metal-based fragments (Au/Au homobimetallic complexes) or by grafting a free bipyridine ligand available for further selective chelation of metals (Au/Cu and Au/Ru heterobimetallic complexes). on a Au-NHC complex The two families of obtained bimetallic complexes are depicted in Figure 5.

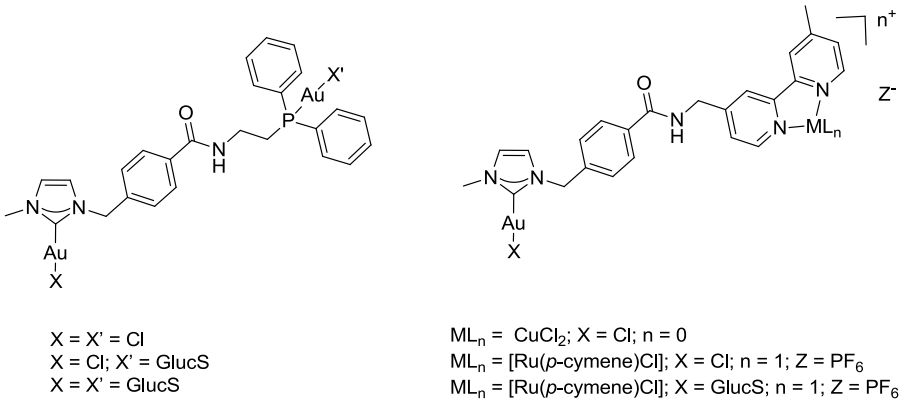



**Figure 5:** Structure of the bimetallic complexes studied in chapter 3.

Subsequently, I performed some preliminary cytotoxicity studies *in vitro* on human ovarian cancer cells and on a model of healthy kidney cells. The introduction of sugar moieties in the Au/Au homobimetallic scaffold did not seem to improve the toxicity against the ovarian cancer cell line but decreased the toxicity against the model of healthy kidney cells *in vitro*. The impact of the sugar moiety was more noticeable in case of the Au/Ru heterobimetallic complex where it seemed to increase both the toxicity and the selectivity toward ovarian cancer cells *in vitro.* Moreover, the Au/Cu bimetallic complexes appeared more toxic than the Au(I) fragment alone and more selective than the Cu(II) fragment. Although it should be mentioned that all our $IC_{50}$ values were obtained in a limited number of experiments and should be confirmed in future experiments, we could observe in most cases an increase in selectivity compared to the mononuclear species both separately and mixed in a 1:1 ratio. I thus confirmed the substantial impact of the association of different metal centers in a single molecular entity as noticed previously for early/late bimetallic complexes.[8]

I also explored the concept of "bifunctionality" in **chapter 6**. I coupled the gold(I) cation to lansoprazole (Figure 6), well-known for its activity as proton-pump inhibitor. Lansoprazole presents a benzimidazole core with nitrogen-donor atoms, which we used for the coordination of the Au(I) center.

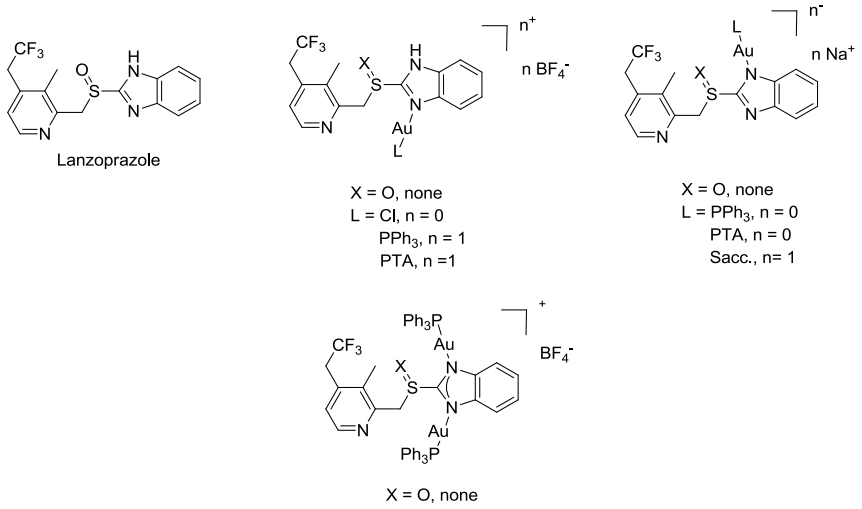



**Figure 6:** Structures of the bifunctional coordination Au(I) complexes studied in chapter 6.

The two lansoprazole-based bimetallic complexes showed similar cytotoxicity in the two tested cancer cell lines ($IC_{50} \approx 1$ μM). It may be possible that the gold-lansoprazole derivatives may still partly act as PPIs, as our results showed an increase of intracellular ATP content in cells treated with the lansoprazole-based compounds with respect to controls as already reported for other PPIs.[10] However, a study on 2-arylimidazole analogs of these compounds identified poly(ADP-ribose) polymerase 1 (PARP-1), known as the "guardian angel" of DNA, as a possible target of these gold complexes.[11] In that case, the biological properties were attributed to the gold cation, so it is likely that the lansoprazole-based gold compounds possess similar properties against PARP-1. Similarly, TrxR could be another possible target of these gold(I) complexes. Therefore the ability of these lansoprazole-based gold complexes to inhibit TrxR and PARP-1 deserves further study. Moreover, the mode of interaction of the compounds with the enzymes could be investigated by high resolution mass spectrometry techniques and biochemical assays in order to determine if the lansoprazole-based ligand influences the biological properties of the gold cation. Those data would enable a better assessment of the contributions of each part of the compounds to the observed cytotoxicity.

To conclude, organometallic gold-based compounds are promising candidates for anticancer chemotherapy due to their higher stability in physiological milieu and, in some cases, their selective toxicity for cancer cells compared to healthy cells. From the twenty synthesized gold-based compounds, two of them (the caffeine-based compound TMX-Au-TMX and the cyclometalated compound with the phosphane-coumarin ligand) are

particularly promising drug candidates. Three potential intracellular targets were identified for gold-based compounds: PARP-1, TrxR and DNA G-quadruplexes. In general, in order to be able to further develop metal compounds as anticancer agents and to optimize the drug design, more mechanistic investigation will be necessary using molecular pharmacological assays coupled to bioanalytical methods.

Moreover, the development of metal-based drug candidates requires toxicity studies to evaluate their possible side effects. Although *in vitro* screening on cell lines is a very interesting tool to rapidly screen a large range of compounds, we have to keep in mind that it is only a model. Within this frame, *ex vivo* models as precision-cut tissue slices (PCTS) described in **chapter 2** appear particularly promising, not only to assess toxicity in healthy tissue, but when prepared from human cancer tissue, they may represent a more relevant model for human tumors. Finally, the evaluation of the most promising compounds *in vivo* is necessary to provide a picture of the real impact of the compounds on both the targeted disease and the healthy organism.

## 2/ Perspectives

I found [TMX-Au-TMX].$BF_4$ to be selective for an ovarian cancer cell line as well as to be a highly selective stabilizer of the G-quadruplex structure of DNA. However, no direct correlation between those two observations have been drawn yet. Thus, understanding much deeper its actual mechanism of action, including its mode of uptake and its distribution into the cells, would provide interesting information to optimize its anticancer properties. To do so, I tried to couple the caffeine scaffold to a coumarin fluorophore without success. This way should still be explored by replacing the coumarin by another fluorophore or by increasing the size of the linker. By tuning the size of the linker we could address the different issues we noticed with that family of compound: quenching of the fluorescence of the coumarin due to close proximity with the NHC moiety as I observed in **chapter 4**, facilitate the synthetic work by increasing the solubility in organic solvents and tune the lipophilicity to optimize its biological properties as demonstrated by McKeage, Berners-Price *et al.*[12] Moreover, the understanding of its interaction and selectivity for the quadruplex structure would be necessary to design more efficient compounds. Concerning the imidazole/coumarin compounds, I obtained relatively contradictory results: the best inhibitor of TrxR appeared to be the less cytotoxic. The lack of activity of that compound could be due to a very low uptake. This question could be solved by quantification of the intracellular gold using ICP-MS. Parallely, a way to increase the uptake of the compound should be developed, such as encapsulation into a liposome.[13]

Because of the promising results we obtained with the phosphane-containing cyclometalated Au(III) complexes, more investigations would be required on their mode of action including transport mechanism, identification of intracellular targets as well as their

electrochemical behavior. Moreover, intracellular distribution studies by fluorescence microscopy are ongoing in the lab using the fluorescent phosphane-based complexes. These questions elucidated, the structure could be optimized using the remaining chlorido ligand for further functionalization.

In the same way, very promising results have been observed in the bimetallic series on both the homo- and heterobimetallic families. However, the majority of them need to be confirmed. Moreover, more cell lines should be tested to have a better view of their potential. I also need to identify the intracellular targets of these complexes. I could observe the importance of the ligands on the gold moiety for both the toxicity and the selectivity for cancer cells, so optimization of these properties could be achieved by working on the coordination environment on the second metal. I can envisage the replacement of the arene ligand on ruthenium as well as replacement of the chlorido ligands on copper by less fairly hydrolysable ligands such as carboxylate or bipyridine/terpyridine-based ligands. Using the presented system, the scope of the second metal could be enlarged to include luminescent metals to allow tracking the compounds *in vitro* in cells via fluorescence microscopy. Moreover, the use of a radiometal such as $^{99m}$Tc could be envisaged for the tracking of the compound *in vivo.*

To better understand the intracellular mechanisms of the lansoprazole-based, interactions of the compounds with reported targets of gold(I) complexes such as PARP-1 and TrxR should be studied. These data should enable us to finer determine the implication of each part (ligand and metal cation) into the biological activity.

Based on these results, I could imagine some promising structures for the development of selective anticancer drugs. The use of bimetallic complexes incorporating thioglucose moieties seems particularly relevant. Moreover, the use of the caffeine scaffold to build up Au(I)-NHC complexes could provide an additional selectivity for cancer cells. Some examples of such caffeine-based bimetallic complexes are depicted in Figure 7.

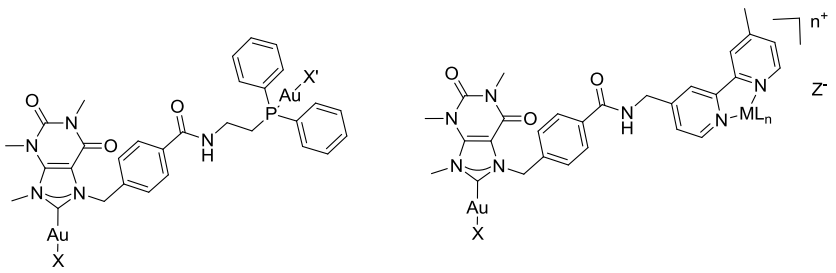



**Figure 7**: Examples of possible caffeine-based bimetallic complexes.

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